

# Zygoty and Placental Anatomy in 15 Consecutive Sets of Spontaneously Conceived Triplets

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**Most triplets are trizygotic because they result from assisted reproduction. Prognosis is generally good. We analyzed 15 sets of triplets who were conceived spontaneously. Six sets were monozygotic, 7 were dizygotic, and only 2 sets were trizygotic. Considered as 45 twin pairs, 25 pairs (56%) were monozygotic. Twenty percent of these twins died as a result of twin-twin transfusion. Spontaneously conceived triplets have high risks compared with those resulting from assisted reproduction. These risks result from a high proportion of monozygotic embryos, many of whom have monochorionic placentas with vascular anastomoses, causing twin-to-twin transfusion.**

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**KEY WORDS:** triplets, zygoty, diseases in triplets, diseases in twins

## INTRODUCTION

It is reported that the outcome of triplet pregnancies is improving [Lipitz et al., 1989]. In part, this results from improved pre-natal care and the prevention of premature onset of labor. However, an important factor is that most higher order multiple pregnancies currently are the products of various types of assisted reproduction for infertility. In one series [Weissman et al., 1991], 24 sets were induced and only 5 were spontaneously conceived. Derom et al. [1991] have shown a 10% frequency of monozygotic (MZ) fetuses among twin pregnancies resulting from assisted reproduction. It might be expected that the frequency of MZ twins and triplets in spontaneously conceived triplets would be higher than 10%. Furthermore, many of such MZ twins

would be monochorionic, with the attendant risks of vascular complications.

Surprisingly few series have been reported in which chorionicity of spontaneously conceived triplets is well documented, yet twin-to-twin transfusion is well known in triplets [Sakala, 1986; Pons et al., 1990]. Indeed, Gonen et al. [1990] reported the outcome of 5 sets of triplet gestations complicated by fetal death. In 4 sets, placentation was monochorionic, triamniotic, while the fifth set was trichorionic. In a larger series, Borlum [1991] reported 15 triplet sets with one or more fetal deaths. Four sets were monochorionic and 6 were dichorionic, triamniotic, implying the presence of a pair of monochorionic twins; thus, 67% of their sets contained at least one pair of monochorionic, monozygotic twins. There was a 67% mortality in the monochorionic triplets, 50% mortality in the dichorionic triplets, and 40% mortality in the trichorionic triplets.

Spontaneously conceived triplets are a special category of triplets with high rates of monozygosity who are at risk for complications of monochorionic placentation, evidence for which should be carefully sought by obstetric ultrasound.

MZ spontaneously conceived triplets with mixed monochorionic and dichorionic placentation offer good opportunities to study possible mechanisms of spontaneous triplet formation at different times after fertilization (see accompanying paper) [Machin, 1996].

The purpose of this paper is to re-emphasize the difference between spontaneously conceived triplets and triplets born from assisted reproduction, since spontaneously conceived triplets contain large numbers of monochorionic MZ twins. In addition, the data are given on zygoty, determined by analysis of highly polymorphic DNA markers in placental tissues. We found a high prevalence of MZ twins and triplets in this series.

The existing literature on chorion status in spontaneously conceived triplets is also reviewed.

## MATERIALS AND METHODS

Twins and higher order multiple pregnancies born at University of Alberta Hospitals are routinely tested for zygoty at birth after sampling of cord, amnion, and chorion from the appropriately labelled placentas. DNA is extracted using standard methods, and zygoty is determined using variable number tandem repeat probes [Higgs et al., 1986; Bell et al., 1981; Nakamura

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et al., 1987]. Among these multiple pregnancies, we tested 15 consecutive sets of spontaneously conceived triplets.

## RESULTS

Results of chorionicity and zygosity analysis are shown in diagrammatic form (Fig. 1) and in Table I. Of the 15 sets, 6 were MZ, 7 were dizygotic (DZ), and 2 were trizygotic (TZ). Thus, 45 embryos were formed from 26 zygotes, at a rate of 1.7 embryos per zygote. The 15 triplet sets contain 45 twin pairs, of which 25 pairs (56%) were MZ. Thirteen of the 25 MZ pairs (52%) were monozygotic, while 38% of dichorionic twins were MZ (Table II). Among the 25 MZ twin pairs, 17 were female and 8 were male. Among the 13 monozygotic twin pairs, 8 were female and 5 were male.

Of the 6 MZ triplet sets, 2 were monozygotic, triamniotic; 3 were dichorionic, triamniotic; and 1 was trichorionic, triamniotic. The 3 sets of MZ triplets born at 24 weeks gestation each contained a pair of monozygotic twins dying of twin-to-twin transfusion, the third triplet (dichorionic in two cases) dying of pre-term complications, but not being involved in the process of twin-to-twin transfusion. All the triplets dying of twin-to-twin transfusion were female, and there were only two sets of male MZ triplets.

Overall perinatal mortality was 9/45 (20%). Mortality was limited to the MZ sets, in which mortality was 50%.

Two sets of female MZ triplets had combined monozygotic and dichorionic placentation, implying consecutive earlier (dichorionic) and later (monozygotic)

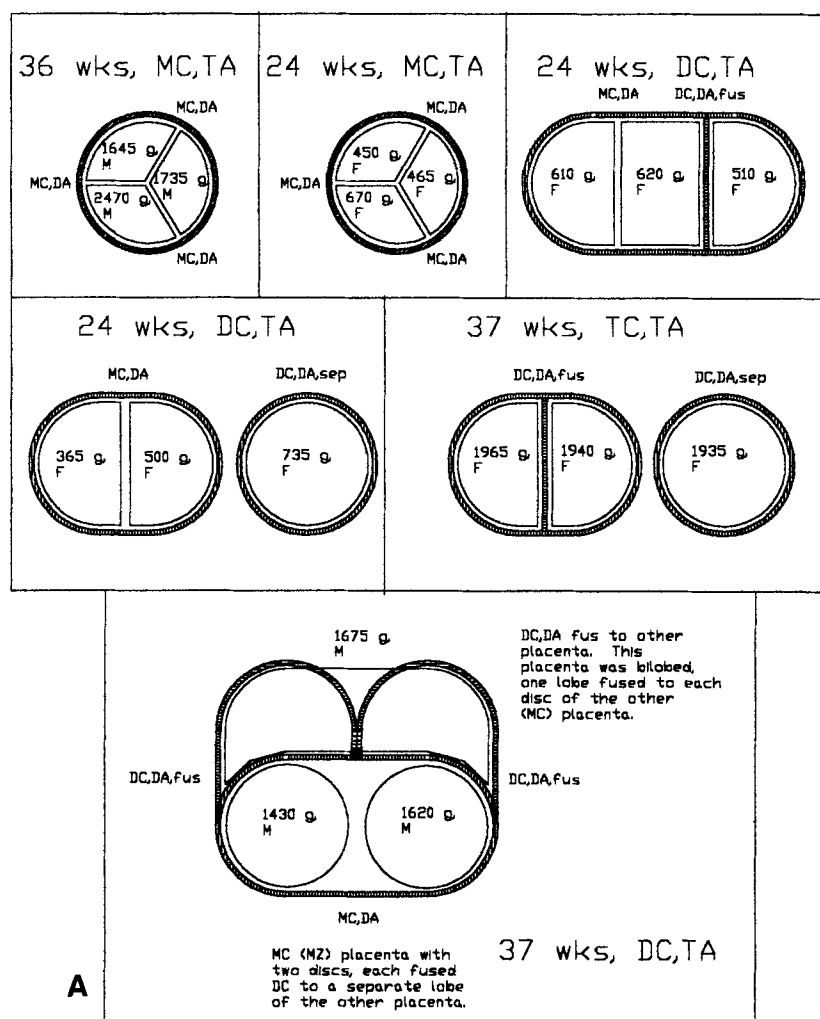


Fig. 1. Diagrammatic representation of zygosity and chorionicity in 15 sets of spontaneously conceived triplets. **A:** 1.1 The 6 sets of MZ triplets. **B:** 1.2 The 7 sets of DZ triplets. **C:** 1.3 The 2 sets of TZ triplets. Gestational age is in weeks. Birth weights and sexes are shown in the placental zone of each triplet. Chorionic membrane is shown thick, hatched. Amniotic membrane is single, thin line within the chorion. In the monozygotic sets, case 6 had an unusual placental anatomy. The dichorionic placenta was bilobed, and each lobe was fused to one of two discs of the monozygotic placenta. Monozygotic placentas rarely have two discs. MZ, monozygotic; DZ, dizygotic; TZ, trizygotic; MC, DA, monozygotic, diamniotic; DC, DA, dichorionic, diamniotic; fus, fused; sep, separate; M, male; F, female.

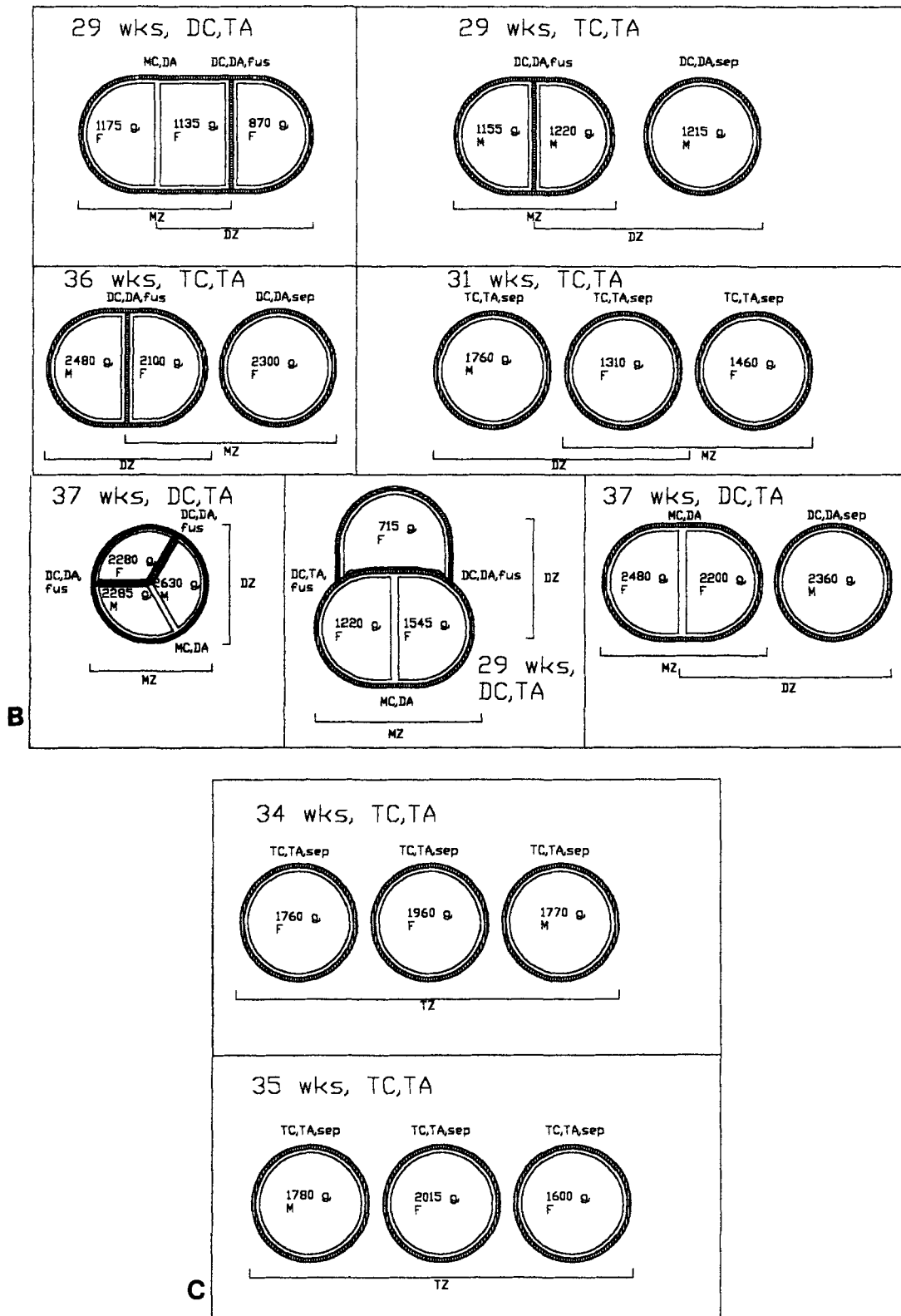


Fig. 1.

TABLE I. Chorionicity and Zygosity in the 15 Sets of Spontaneously Conceived Triplets

	MZ	DZ	TZ	Total	
				n	Percent
Monochorionic	2	0	0	2	13
Dichorionic	3	4	0	7	47
Trichorionic	1	3	2	6	40
Total	6	7	2	13	100

twinning events. These sets were investigated for pattern of X-inactivation [Bamforth et al., 1996].

Vascular anastomoses were present in most monochorionic twins. In the 3 cases of severe pre-natal twin-to-twin transfusion, arterio→venous anastomoses from donor to recipient were documented. In the case of twin-to-twin transfusion with monochorionic, triamniotic placentation, there was also an arterio→arterial anastomosis between the unaffected triplet and the donor. In the monochorionic, triamniotic triplets born at 36 weeks, there were arterio→arterial anastomoses between all 3 triplets. The triplets survived intact (Fig. 2). In the dichorionic, triamniotic DZ triplets delivered at 29 weeks, there were no anastomoses between the monochorionic twins. Similarly, in the dichorionic, triamniotic triplets born at 37 weeks, no anastomoses were identified between the monochorionic twins.

In the 2 dichorionic MZ pairs, the relative efficiency of placental function could not directly be compared between the monochorionic and dichorionic placentas. Body weights of the twins were affected by pre-natal and peri-natal twin-to-twin transfusion, and the sites of implantation of the two placentas were not known.

### LITERATURE REVIEW

Large series of triplets are currently published in which no distinction is made between spontaneously conceived triplets and products of assisted reproduction. In many, chorionicity is not documented. The older literature, dating from before 1985, can be assumed to consist largely of spontaneously conceived triplets. This literature is summarized in Table III. It is notable that the discussion of spontaneously conceived triplets by Boyd and Hamilton [1970] wrongly assumes that triplets who are dichorionic or trichorionic in relation to each other are also DZ or TZ.

This literature review shows that most spontaneously conceived triplets contain at least a pair of monochorionic MZ twins, and that 24% are actually monochorionic triplets.

TABLE II. 15 Sets of Spontaneously Conceived Triplets Analyzed as 45 Twin Pairs for Chorionicity and Zygosity

	MZ		DZ		Total	
	n	Percent	n	Percent	n	Percent
Monochorionic	13	29	0	0	13	29
Dichorionic	12	27	20	44	32	71
Total	25	56	20	44	45	100

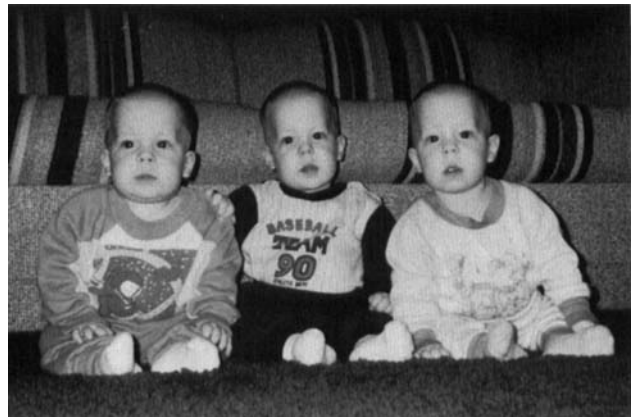


Fig. 2. Monochorionic, monozygotic triplets at age 6 months.

This review omits the series of Nigerian triplets reported by Nylander [1971] which, as would be expected, contains a larger proportion of dichorionic and trichorionic triplets. Also omitted are Japanese series in which an excess of monochorionic MZ twins and triplets would be expected [Imaizumi and Inouye, 1980; Matayoshi and Yoshida, 1989]. Derom et al. [Derom, personal communication, 1995] analysed zygosity, chorionicity, and sex ratios in 31 spontaneously-conceived triplet sets. Of these, 6 (19%) were MZ, 17 (55%) were DZ, and only 8 (26%) were TZ. Considered as 91 twin pairs, 35 (38%) were MZ and 58 (62%) were DZ. There was an excess of females among the MZ triplets.

### DISCUSSION

Chorionicity and zygosity are seldom reported in triplet studies. Allen [1960] used the Hardy-Weinberg formula to calculate triplet and quadruplet zygosity; he predicted the ratio of MZ:DZ:TZ triplets to be 1:2:1 in whites and 1:3:2 in blacks. In this series of 15 consecutive sets of spontaneously conceived triplets, we found that a significant proportion were MZ, with monochorionic, dichorionic, or trichorionic placentation. Even among the 6 trichorionic triplet sets, 4 contained MZ twins or triplets. Three sets of triplets died of consequences of twin-to-twin transfusion.

If the 15 triplet sets are analyzed as 45 twin pairs, this series shows a high incidence of monochorionic twins and MZ twins (Table II). The proportion of MZ twins with dichorionic placentation is far higher in spontaneously conceived triplets than in spontaneous twin sets (Table IV), 27% compared with 10%. A comparison of outcomes in 394 induced and spontaneous triplets [Hendricks and Newman, 1992], is shown in Table V. Absolute numbers in the two groups are not given. The lower mean birth weight in spontaneously conceived triplets was statistically significant.

Because spontaneously conceived triplets are a subgroup of triplets containing a high proportion of MZ twins and triplets, they require more intense expectant prenatal management than induced triplets. In this

TABLE III. Literature Review of Chorionicity in 38 Sets of Presumed Spontaneously Conceived Triplets

	Trichorionic		Dichorionic		Monochorionic	
	n	Percent	n	Percent	n	Percent
Potter and Fuller [1949]	1	25	2	50	1	25
Boyd and Hamilton [1970]	1	12	3	38	4	50
Corney et al. [1975]	3	60	1	20	1	20
Williams [1926]	1	25	3	75	0	0
Carter [1956]	0	0	1	50	1	50
Present series	6	40	7	47	2	13
Total	12	32	17	45	9	24

TABLE IV. Comparison Between Chorionicity and Zygosity of Twins of Triplet Sets and Spontaneously Conceived Twins (%)\*

	Twins in 15 spontaneously conceived triplet sets			Spontaneous twins		
	MZ	DZ	Total	MZ	DZ	Total
Monochorionic	29	0	29	20	0	20
Dichorionic	27	44	71	10	70	80
Total	56	44	100	30	70	100

\*From Cameron [1970].

TABLE V. Perinatal Outcomes in 394 Induced and Spontaneous Triplet Gestations [Hendricks and Newman, 1992]

	Induced	Spontaneous
Gestational age, weeks (mean)	33.8	33.7
Mean birth weight, g	1,889	1,768
Death from prematurity	10	13
Delivery at <30 weeks	18	15

way, spontaneously conceived triplet sets containing monochorionic twins can be identified. Even among dichorionic triplets, we found significant numbers of MZ twins. There is a general view that most spontaneously conceived triplets are trichorionic and TZ, but results from this series and from the literature review do not support this.

In comparison with spontaneously conceived twins (Table IV), this spontaneously conceived triplet series shows a greater proportion of MZ twins (54% vs. 30%), monochorionic twins (28% vs. 20%), and dichorionic MZ twins (36% vs. 12% of dichorionic twins). In view of the frequent assumption that dichorionic twins are DZ, zygosity testing of spontaneously conceived triplets seems appropriate, in order to ascertain the MZ dichorionic triplets. Spontaneously conceived triplets are not commonly the result of polyovulation and trizygosity.

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